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Lower antibody functionality in middle-aged adults compared to adolescents after primary meningococcal vaccination: Role of IgM

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ABSTRACT

Introduction: Successful vaccination of elderly persons is often hampered by immunological ageing, leaving part of the elderly population vulnerable for infectious diseases. As an alternative, timely vaccinations might be administered at middle-age, before reaching old age. Studies evaluating the immunological fitness of middle-aged adults are warranted. In this study we compared the immunogenicity of a primary meningococcal vaccination in Dutch middle-aged adults with that in adolescents, in order to gain knowledge on the early signs of immune ageing.

Methods: In this study, we compared the antibody responses after a primary meningococcal vaccination between middle-aged adults (50–65 years of age, $N = 204$) and adolescents (10–15 years of age, $N = 225$). Blood samples were taken pre-, as well as 28 days and 1 year post-vaccination. Functional antibody titers were measured with the serum bactericidal killing assay using baby rabbit complement (rSBA). Meningococcal polysaccharide (PS) specific IgG and IgM concentrations were determined with a fluorescent bead-based multiplex immunoassay.

Results: Lower post-vaccination functional antibody titers against meningococcal group W and Y were observed in the middle-aged adults compared to the adolescents. One year post-vaccination, also a significantly higher proportion of the middle-aged adults possessed an rSBA titer below protection level. A large reduction in post-vaccination IgM concentrations was observed in the middle-aged adults, whereas IgG concentrations were only marginally different between the two age groups.

Strong correlations between the post-vaccination rSBA titers and IgM concentrations were found both in the middle-aged adults and the adolescents.

Conclusion: Although protective antibody titers were initiated after primary meningococcal vaccination in middle-aged adults, antibody functionality was significantly lower as compared to that in adolescents. This difference was mainly caused by lower IgM responses. Our results indicate early signs of immune ageing in middle-aged adults, which is important knowledge for the development of future vaccine strategies to better protect elderly persons against infectious diseases.

1. Introduction

Prevention of infectious diseases in the elderly is important to establish healthy ageing in a rapidly ageing world population (Michel and Lang, 2011; UnitedNations, 2015). Yet, effective vaccine responses in the elderly are often hampered by immunological ageing, leaving part of the elderly vulnerable for infectious diseases (Lang and Aspinall, 2012). Timely vaccination of middle-aged adults, instead of elderly persons, may be a solution to strengthen the memory immunity before reaching old age (Michel and Lang, 2011; Rappuoli et al., 2011). However, the immunological fitness of the middle-aged adults is not well-defined.

Comparison of vaccine immunogenicity in middle-aged adults with that in younger age groups is a valuable method in order to gain knowledge on the early signs of immune ageing. Notwithstanding, comparative studies are often biased by differences in pre-vaccination

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immunity between age groups, affecting the vaccine induced responses. This pre-vaccination immunity is frequently different between old and young participants due to differences in vaccine history or natural exposure (Furman et al., 2013a, 2013b; Weinberger et al., 2013). Therefore, *de novo* vaccine antigens should be used to describe differences in vaccine responses between young and old (Michel and Lang, 2011; Lang and Aspinall, 2012).

In order to compare the vaccine immunogenicity between middle-aged adults and a young age group, a primary tetravalent meningococcal vaccination is employed, containing the meningococcal groups A,C, W, and Y. Historical circulation of meningococci W (MenW) and meningococci Y (MenY) in the Netherlands has been low (de Voer et al., 2010), indicating that the vaccination will most likely induce primary vaccine responses in both age groups. Of note, meningococci C (MenC) is given as a booster response to the adolescents and therefore vaccine responses cannot be compared between the age groups. Also comparison of meningococci A (MenA) is difficult due to interference of cross-reactive antibodies in the assays. Meningococcal vaccine immunogenicity is highly studied in young children and adolescents, whereas immunogenicity studies in older adults are scarce.

The highest numbers of meningococcal cases are observed in young children (< 5 years) and adolescents. However, a peak of meningococcal cases is also seen in older adults (65 +), mainly caused by the MenW and MenY (Diseases, 2005; Stoof et al., 2015; Edge et al., 2016). During the currently ongoing MenW outbreak, a large proportion of the cases is seen in the oldest age group (RIVM, 2017). Moreover, the highest case fatality rate by meningococci is observed in the elderly (Stoof et al., 2015).

We previously showed that a primary tetravalent meningococcal vaccination induced protective, bactericidal antibody titers against meningococcal group C, W, and Y in middle-aged adults (50–65 years of age) that lasted for at least one year (Heiden et al., 2017). Moreover, we demonstrated that the protective antibody titers against the *de novo* antigens MenW and MenY were highly correlated with the meningococcal specific IgM responses and that these IgM responses decreased with age even in the limited age range of our study cohort (Heiden et al., 2017).

In this study, the immunogenicity of a primary meningococcal vaccination was compared between middle-aged adults and adolescents (10–15 years of age) (van Ravenhorst et al., 2017a, 2017b). As expected, the meningococcal groups W (MenW) and Y (MenY) initiated a clear primary immune response in the majority of the participants and hence these meningococcal groups were used for comparison.

2. Methods

2.1. Study design and participants

This study combines data from two different phase IV single center and open-label studies. Both studies assessed the immunogenicity of a primary tetravalent meningococcal vaccine conjugated to tetanus toxoid (MenACWY-TT, Nimenrix, GlaxoSmithKline) either in adolescents or in middle-aged adults (Heiden et al., 2017; van Ravenhorst et al., 2017a, 2017b). The adolescents were vaccinated and sampled in the spring of 2014 and the middle-aged adults in the autumn of 2014. Detailed exclusion criteria of both studies have been previously described (Heiden et al., 2017, van Ravenhorst et al., 2017a, 2017b). In short, both the adolescents and middle-aged adults were excluded when they showed signs of acute illness at the time of vaccination, used immune suppressive medication, had allergies to vaccine components, had a history of meningococcal disease or were administered a previous tetravalent meningococcal vaccination. Written informed consent was obtained from all participants and all procedures were in accordance with the Declaration of Helsinki. The medical ethical committee: Medical Research Ethics Committees United (MEC-U) approved the studies and both studies were registered at the Dutch trial register (adolescents: NTR4430; middle-aged adults: NTR4636).

2.2. Vaccination and blood sampling

A pre-vaccination blood sample was taken from all participants before intramuscular administration of the tetravalent meningococcal vaccine conjugated to tetanus toxoid vaccine (MenACWY-TT; Nimenrix). Subsequently, blood samples were drawn at 28 days and at 1 year post-vaccination. Serum samples were collected using serum clotting tubes (BD Biosciences) and were stored at − 20 °C until further use.

2.3. Serological analysis

Serological analyses were performed as previously described (Heiden et al., 2017; van Ravenhorst et al., 2017a, 2017b). In short, MenW and MenY PS-specific IgG and IgM concentrations were determined with the fluorescent bead-based-multiplex immunoassay (MIA) (de Voer et al., 2009; Heiden et al., 2017; van Ravenhorst et al., 2017a, 2017b). The MenW and MenY serum bactericidal antibody titers were assessed using baby rabbit complement (rSBA) (Pelfreez, LOT#13035EL) and the MP01240070 (MenW) and S-1975 (MenY) strains, kindly donated by Prof. Dr. Ray Borrow from the Vaccine Evaluation Unit at Manchester (PHE). The rSBA titer was defined as the highest serum dilution yielding ≥ 50% killing after 60 min of incubation at 37 °C (Maslanka et al., 1997). The internationally accepted level of protection used was an rSBA titer ≥ 8, whereas a titer of ≥ 128 was used as a more conservative protection level (Borrow et al., 2001). Participants with an rSBA titer below the detection level of the assay were considered seronegative, and were given an rSBA titer of 2 for statistical purposes (Borrow et al., 2001; Borrow et al., 2005).

2.4. Statistics

Prior to all analyses, normal distribution of the data was checked. The geometric mean rSBA titers (GMTs) and IgM/IgG concentrations (GMCs) with the 95% confidence intervals (95% CI) were presented. The pre-vaccination rSBA GMTs as well as the pre-vaccination IgM and IgG GMCs were compared with the Mann Whitney *U* test between the adolescents and middle-aged adults. The 28 days and 1 year post-vaccination rSBA titers and IgM and IgG concentrations were log-transformed to reach a normal distribution of the data. All post-vaccination responses were compared between the adolescents and middle-aged adults using linear regression, with adjustment for pre-vaccination values.

Moreover, the proportion of participants with an rSBA titer ≥ 8 and ≥ 128 was calculated with the Wilson/Brown test and compared between the two groups with the Chi-squared test. The increase in rSBA titers at 28 days post-vaccination was determined as: rSBA titer 28 days/rSBA titer pre-, whereas the antibody decay was determined as: rSBA titer 1 year/rSBA titer 28 days.

All IgM and IgG analysis were performed on the total group of participants as well as only on participants with an undetectable pre-vaccination rSBA titer (rSBA = 2).

The correlations between the IgM and IgG concentrations with the rSBA titers were determined with the Pearson's correlation test.

Graphpad Prism V7 and SPSS V22.0 were used for the statistical analysis. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Participant characteristics

In total, blood samples from 225 adolescents (10–15 years of age) and 204 middle-aged adults (50–65 years of age) were analyzed for PS-specific IgG and IgM. The functional antibody titers (rSBA titers) were

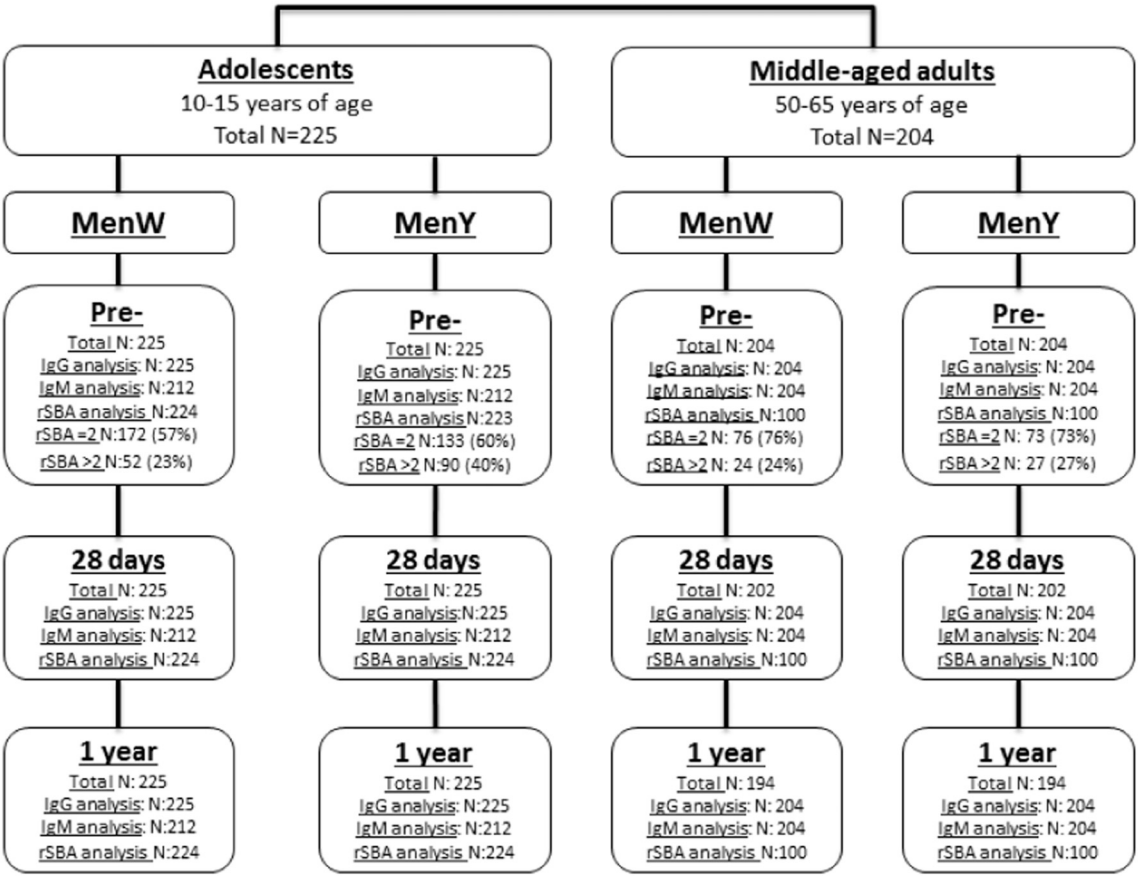

Fig. 1. Cohort outline.

determined in all adolescent samples and in 100 middle-aged participants, as previously described (Heiden et al., 2017; van Ravenhorst et al., 2017a, 2017b). Since rSBA titers were similar in the comparison of the first 50 and second 50 middle-aged participants, these 100 middle-aged adults have proven to be representative for these age group. An overview of the samples used in this comparative study is depicted in Fig. 1.

3.2. Lower MenW and MenY rSBA titers in middle-aged adults compared to adolescents

Protective pre-vaccination rSBA titers (> 8) were found in a part of participants (MenW: A: 15%, M: 23%, MenY: A: 32%, M: 27%) (Table 1). Although low in both age groups, the pre-vaccination rSBA geometric mean titers (GMTs) for MenY were significantly lower in the middle-aged adults than in the adolescents (p-value 0.046; Table1). No significant difference in pre-vaccination rSBA titer was found for MenW between the two age groups (p-value 0.728; Table 1).

At 28 days post-vaccination, significantly lower GMTs were found in the middle-aged adults for both meningococcal groups (p-values < 0.001; Fig. 2a–b and Table 1), which was also reflected in a significantly lower antibody increase 28 days post-vaccination in the middle-aged adults as compared to the adolescents (MenW p-value < 0.001 and MenY p-value = 0.008; Table 1). However, this lower antibody response after 28 days did not result in a lower percentage of the middle-aged adults reaching the rSBA protection titer of 8 (MenW p-value = 0.599 and MenY p-value = 0.054; Table 1). Only a slightly lower percentage of the middle-aged participants reached the more conservative protection level of 128 for MenY (p-value = 0.018; Table 1) than the adolescents.

At 1 year post-vaccination, a significantly lower percentage of the middle-age adults possessed an rSBA titer ≥ 8 against MenW (p-value = 0.018) and MenY (p-value < 0.001) (Fig. 2a-b, and Table 1). Next to the lower antibody increase 28 days post-vaccination, also a significant higher antibody decay was found in the middle-aged adults for MenY after 1 year (p-value < 0.001) but not for MenW (p-value = 0.484; Table 1).

The differences between the middle-aged adults and the adolescents were slightly enlarged when comparing the pre-vaccination seronegative persons only, at one month and one year post-vaccination (Fig. 2c and d).

3.3. Lower MenW and MenY specific IgM responses in the middle-aged adults

Although low in both groups, significantly lower pre-vaccination IgM concentrations were found for both MenW and MenY in the middle-aged adults as compared to the adolescents (p-values < 0.001) (Fig. 3a–b). After adjusting for the differences in pre-vaccination concentrations, significantly lower post-vaccination IgM responses for MenW and MenY were observed in the middle-aged adults than in the adolescents (p-values < 0.001; Fig. 3a–b) both at 28 days and 1 year. Similar results were obtained when only participants without detectable pre-vaccination rSBA titers (rSBA = 2) were analyzed (Fig. 3c–d). The geometric mean IgM concentrations at the different time points are depicted in Supplementary Table 1.

3.4. Lower MenW and MenY specific IgG responses in middle-aged adults

Pre-vaccination, the middle-aged adults had significantly lower meningococcal specific IgG concentrations than the adolescents, for both MenW and MenY (p-values < 0.001; Fig. 4a–b). After

Table 1
Comparison of the MenW and MenY rSBA titers in the adolescents and middle-aged adults.

		MenW					MenY				
		Adolescents		Middle-aged		*p*-value	Adolescents		Middle-aged		*p*-value
Pre-											
GMT	**[95% CI]**	4.3	[3.4–5.4]	5.4	[3.8–7.8]	0.728	10.7	[7.8–14.6]	6.9	[4.5–10.4]	**0.046**
% rSBA ≥ 8	**[95% CI]**	15.2	[11.1–20.5]	23.0	[15.8–32.2]	0.088	32.3	[26.5–38.7]	27.0	[19.3–36.4]	0.341
% rSBA ≥ 128	**[95% CI]**	11.0	[7.6–15.7]	16.6	[10.0–23.6]	0.186	30.0	[24.4–36.4]	17.0	[10.9–25.5]	**0.013**
28 days											
GMT	**[95% CI]**	5790	[4829–6941]	1687	[1252–2272]	< **0.001**	3954	[3437–4550]	1448	[1026–2044]	< **0.001**
% rSBA ≥ 8	**[95% CI]**	98.2	[95.5–99.3]	99.0	[94.6–99.9]	0.599	99.6	[97.6–100]	97.0	[91.5–99.2]	0.054
% rSBA ≥ 128	**[95% CI]**	98.2	[95.6–99.3]	97.0	[91.5–99.1]	0.484	99.1	[96.8–99.8]	95.0	[88.8–97.8]	**0.018**
1 year											
GMT	**[95% CI]**	1165	[1006–1349]	330.8	[234.8–466.2]	< **0.001**	1331	[1114–1591]	247.3	[158.1–386.7]	< **0.001**
% rSBA ≥ 8	**[95% CI]**	98.7	[96.1–99.6]	94.0	[87.5–97.2]	**0.018**	97.8	[94.9–99.1]	86.0	[77.9–91.5]	< **0.001**
% rSBA ≥ 128	**[95% CI]**	98.7	[96.1–99.6]	88.0	[80.2–93.0]	< **0.001**	96.9	[93.7–98.5]	79.0	[70.0–85.8]	< **0.001**
Increase 28 days	**[95% CI]**	1384	[1074–1783]	311	[200–485]	< **0.001**	393	[287–538]	209	[126.6–345]	**0.008**
Decay 1 year	**[95% CI]**	0.20	[0.18–0.23]	0.19	[0.15–0.25]	0.484	0.33	[0.28–0.38]	0.16	[0.12–0.22]	< **0.001**

The pre-vaccination geometric mean titers (GMTs) between the two age groups were compared with the Mann Whitney *U* test. The GMTs at 28 days and 1 year post-vaccination were compared using linear regression analysis with adjustment for pre-vaccination titers. The increase in rSBA titers at 28 days post-vaccination was determined as: rSBA titer 28 days/rSBA titer pre-, whereas the antibody decay was determined as: rSBA titer 1 year/rSBA titer 28 days. The increase at 28 days, and the decay 1 year post-vaccination were compared between the adolescents and middle-aged adults with the Mann Whitney U test. The proportions of participants with an rSBA titer above the protection levels of 8 and 128 were compared with the Chi Squared test. Significant differences are given in bold.

adjustments for these differences in pre-vaccination IgG concentrations, significantly lower meningococcal group specific IgG responses were found in the middle-aged adults 28 days post-vaccination for both MenW (*p*-value = 0.001) and MenY (*p*-value = 0.01). This difference persisted until 1 year post-vaccination for MenW (*p*-value = 0.004), whereas, no significant difference was observed in MenY IgG concentrations after 1 year (*p*-value = 0.627) (Fig. 4a–b). Again, approximately similar results were found when only participants without a detectable pre-vaccination rSBA titer (rSBA = 2) were compared (Fig. 4c–d). The geometric mean IgG concentrations are depicted in Supplementary Table 2.

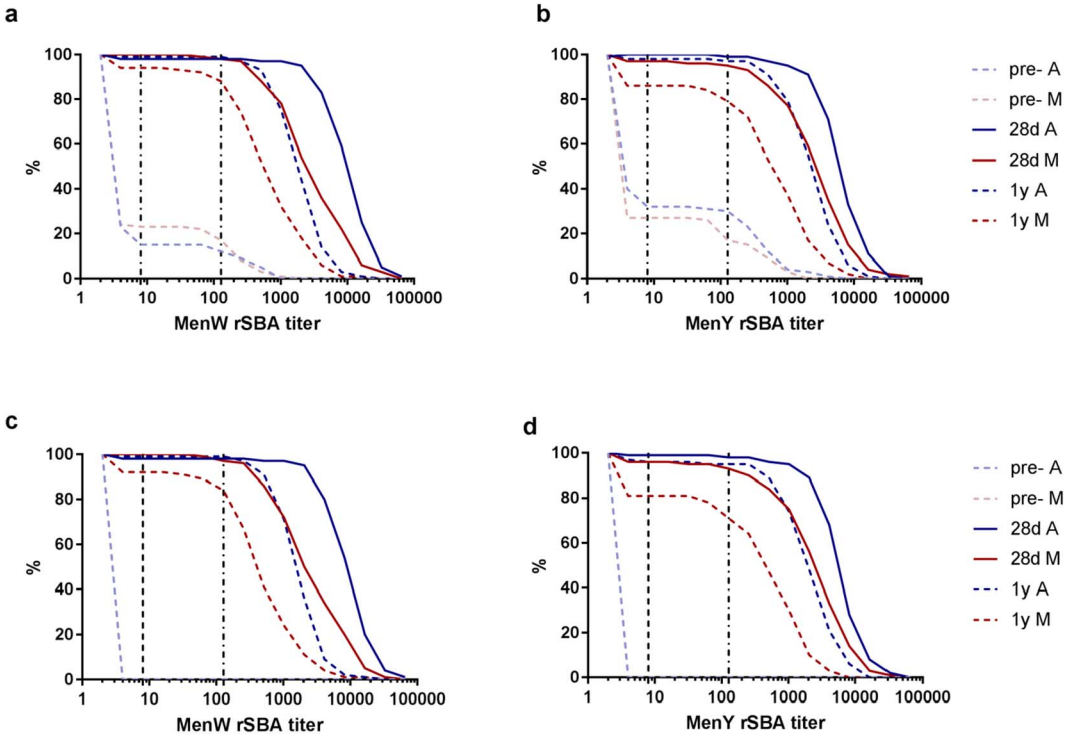

Fig. 2. MenW and MenY rSBA responses in adolescents and middle-aged adults.
Reverse cumulative distribution curves of the rSBA titers at the different time points pre- and post-vaccination for the adolescents (A, blue) and middle-aged adults (M, red) for MenW **(a and c)** and MenY **(b and d).** In panel **a** and **b** all participants (MenW: *N* = 224 A and *N* = 100 M, MenY: *N* = 223 A and *N* = 100 M) are represented whereas, panels **c** and **d** included only participants without detectable pre-vaccination rSBA titers (MenW: *N* = 172 A, *N* = 76 M, MenY: *N* = 133 A and *N* = 73 M). The vertical lines represent the protection lines of rSBA titers 8 and 128 respectively. Pre- = pre-vaccination, 28 d = 28 days post-vaccination, 1y = 1 year post-vaccination.

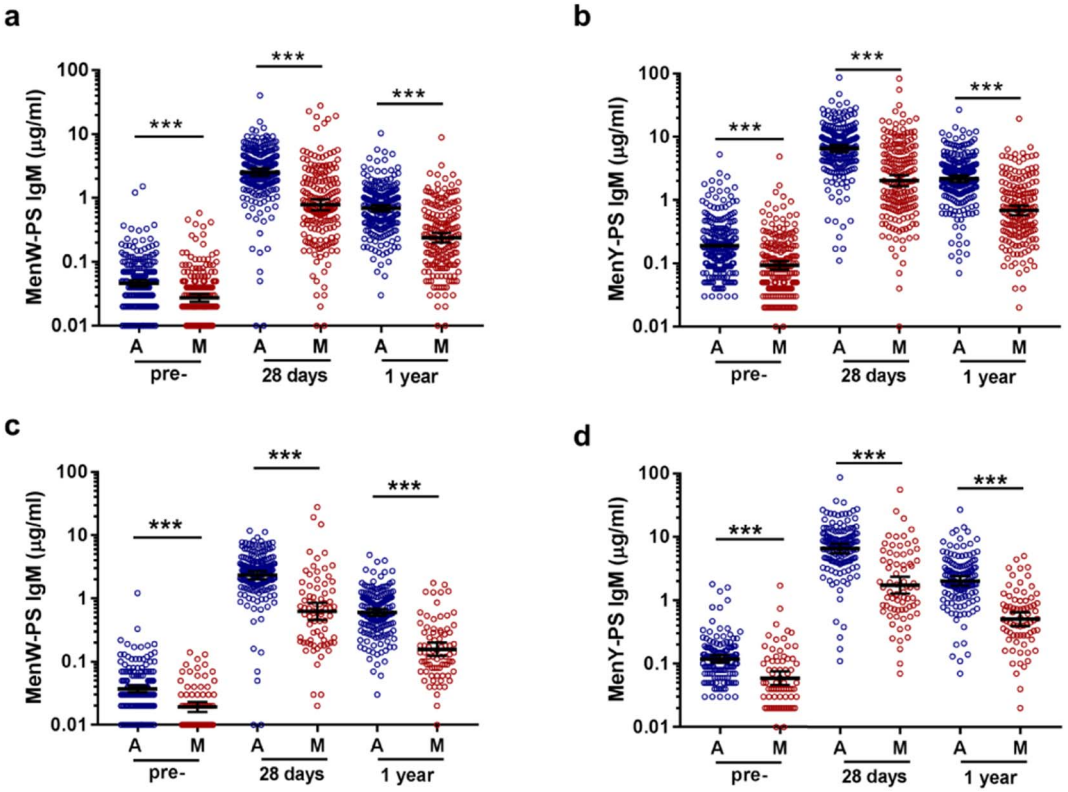

Fig. 3. Lower MenW and MenY PS-specific IgM responses in the middle-aged adults as compared to the adolescents.
Comparison of the MenW **(a and c)** and MenY **(b and d)** PS-specific IgM responses between the adolescents (A,blue) and middle-aged adults (M,red). In **a** and **b** all participants were compared, whereas participants without a detectable pre-vaccination rSBA titer (rSBA = 2) were compared in **c** and **d.** Pre-vaccination IgM concentrations were compared between the adolescents and the middle-aged adults with the Mann Whitney *U* test, whereas IgM concentrations 28 days and 1 year post-vaccination were log transformed, after which linear regression with adjustments for the pre-vaccination IgM concentrations was performed. ***$p < 0.001$.

3.5. Strong correlation between the post-vaccination rSBA titers and IgM concentrations in both the middle-aged adults and the adolescents

Both in the middle-aged adults (MenW: $R = 0.753$, $p < 0.001$; MenY: $R = 0.707$, $p < 0.001$) and adolescents (MenW: $R = 0.801$, $p < 0.001$; MenY: $R = 0.529$, $p < 0.001$) high to moderate correlations were observed between the post-vaccination rSBA titers and the IgM concentrations (Fig. 5a–b). The correlations between the post-vaccination rSBA titers and the IgG concentrations were low to moderate in both the middle-aged adults (MenW: $R = 0.342$, $p < 0.001$; MenY: $R = 0.308$, $p < 0.001$) and the adolescents (MenW: $R = 0.359$, $p < 0.001$; MenY: $R = 0.268$, $p < 0.001$) (Fig. 5c–d). Our findings suggest, as before (Heiden et al., 2017), that the functional antibody titers after primary vaccination are mainly mediated by IgM.

4. Discussion

Within this comparative study, we showed that middle-aged adults possessed lower antibody functionality up to one year after primary meningococcal group W and Y vaccination in comparison with adolescents. At 1 year post-vaccination, a significantly higher percentage of the middle-aged adults showed an rSBA titer below the protection level. These lower bactericidal responses were mainly caused by lower IgM responses in the middle-aged adults, although a slightly lower IgG response was also observed with age.

We are the first to compare the immunogenicity of the conjugated meningococcal vaccine in older adults with that in a younger age group. Of note, information about meningococcal vaccine immunogenicity in middle-aged adults is scarce. However, some studies compared the immunogenicity of the pneumococcal polysaccharide vaccine between young adults (18–30 years of age) and elderly (64–88 years of age). These studies found a reduced capacity in opsonizing pneumococci due to a low IgM response in the elderly participants (Park and Nahm, 2011; Leggat et al., 2013; Westerink et al., 2014), which is highly comparable with our findings after primary meningococcal vaccination. In addition, the pneumococcal vaccine primarily induced switched memory B cells (CD27 + IgM-) in the elderly, whereas young participants mainly showed increased CD27 + IgM + cells (Leggat et al., 2013), likely underlying the drop in IgM response with age. Nonetheless, these authors found similar IgG responses in both age groups, which is not fully in line with our findings of a slightly lower IgG response after primary meningococcal vaccination in the middle-aged adults. This discrepancy might be caused by differences in bacterial circulation between pneumococci and meningococci, resulting in more frequent historical contacts with pneumococci as compared to meningococci. Moreover, the addition of a carrier protein in the meningococcal vaccine likely leads to different cellular immune responses compared to the plain polysaccharides for pneumococci, complicating a head to head comparison.

The lower IgM responses found in the middle-aged adults in our study agree with the previously found decrease in total serum IgM concentrations and numbers of IgM + B–cells during chronological ageing (Wu et al., 2012; Martin et al., 2015; Dunn-Walters, 2016). These lower IgM responses largely affect the antibody functionality at advanced age, since IgM was previously shown to be highly functional in complement binding and subsequently killing of the bacteria (Shyur et al., 1992). This important role for IgM is also confirmed by the sharp drop in rSBA titers after depletion of serum IgM in the middle-aged adults (Heiden et al., 2017). Importantly, these diminished IgM responses with age reduce the capacity to effectively respond to primary bacterial infections or vaccinations. On the contrary, the correlations between the IgG concentrations and rSBA titers were low in both the adolescents and the middle-aged adults, indicating less crucial roles for

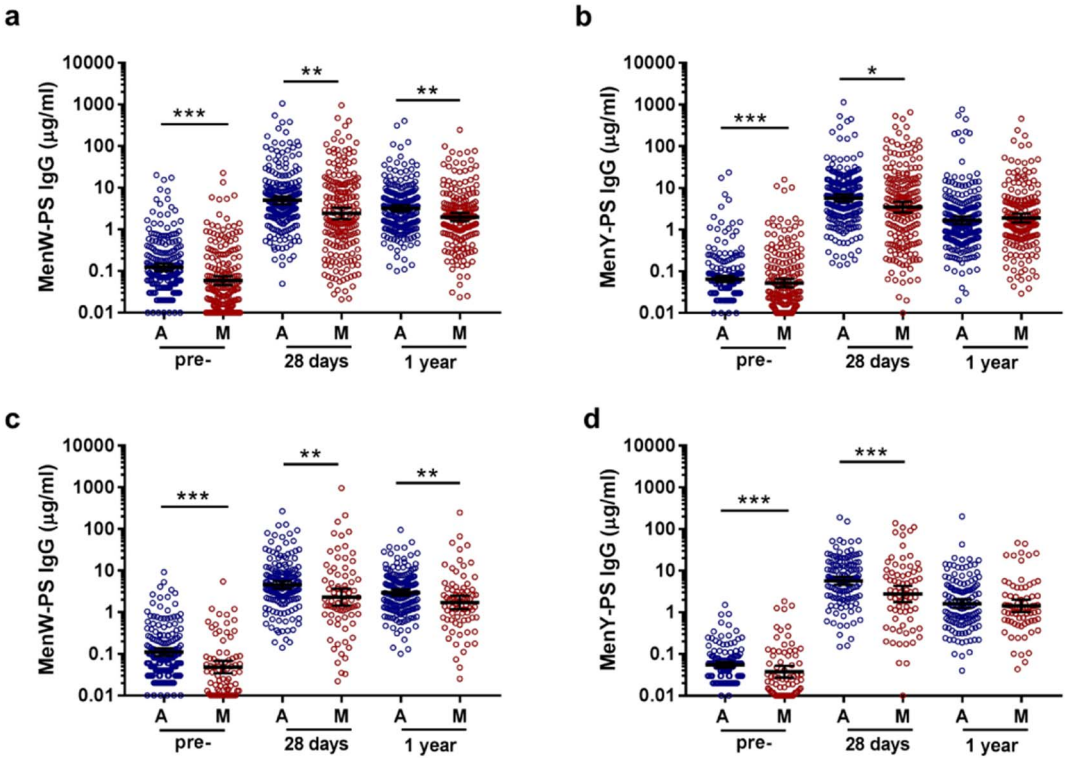

Fig. 4. Lower MenW and MenY PS-specific IgG responses in the middle-aged adults as compared to the adolescents.
Comparison of the MenW **(a and c)** and MenY **(b and d)** PS-specific IgG responses between the adolescents (A,blue) and middle-aged adults (M,red). In **a** and **b** all participants were compared, whereas participants without a detectable pre-vaccination rSBA titer (rSBA = 2) were compared in **c** and **d.** The pre-vaccination IgG concentrations were compared between the adolescents and the middle-aged population with the Mann Whitney U test, whereas IgG concentrations 28 days and 1 year post-vaccination were log transformed, after which linear regression with adjustments for the pre-vaccination IgG titer was performed. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

IgG in response to primary meningococcal vaccination. This notion is totally different after booster vaccinations where strong correlations between the high IgG and rSBA responses were observed (van Ravenhorst et al., 2017a, 2017b) and relatively low IgM concentrations were found (data not shown). These findings suggest that the contribution of IgG to the meningococcal antibody functionality depends on the nature of the vaccine response, being either a primary or booster vaccination.

In addition, we also observed faster antibody decay at 1 year post-vaccination in the middle-aged adults as compared to the adolescents. This finding suggests an age related difference in the formation and survival of long-lived plasma cells, since long-term antibody production is maintained by long-lived plasma cells residing in the bone marrow (Manz et al., 1997). Others previously showed that fat deposition and reduced production of survival factors in the bone marrow led to reduced survival of long-lived plasma cells at older age (Siegrist and Aspinall, 2009; Pritz et al., 2014; Pritz et al., 2015; Pangrazzi et al., 2017). Moreover, the homing of plasma cells towards the bone marrow in order to become long-lived plasma cells was decreased with age (Pritz et al., 2015). Subsequently, our data suggests that the age related reduction in both the numbers of IgM + B-cells and bone marrow survival niches for long-lived plasma cells already affect primary immune responses to meningococcal antigens in middle-aged adults.

Furthermore, the T-cell help, as initiated by the tetanus toxoid carrier protein, might have affected the vaccine responsiveness. This T-cell help may be different between the two age groups, due to a distinct tetanus vaccination history. Moreover, a shift in the T-cell compartment from more naïve to senescent memory T-cells with age, as well as increased numbers of regulatory T-cells (Arnold et al., 2011; den Braber et al., 2012; Herndler-Brandstetter et al., 2013; Akbar et al., 2016), may have diminished the T-cell response towards the carrier protein in the middle-aged adults and subsequently have affected the humoral response, but this is currently unknown.

Remarkably, the differences between the adolescents and middle-aged adults were enlarged when only seronegative participants were compared. These results indicate that booster responses after natural exposure are more immunogenic in middle-aged adults as compared to primary immune responses. This finding is in agreement with others who found large influences of pre-vaccination immunity on vaccine responses in older adults (Furman et al., 2013a, 2013b; Weinberger et al., 2013; Tsang et al., 2014).

Although the IgG and IgM concentrations in both age groups were low before vaccination, we observed significantly lower pre-vaccination IgG and IgM concentrations in the middle-aged adults. This small difference between the two age groups might suggest a higher circulation of meningococcal bacteria in the adolescents group, complying with the general higher meningococcal carriage rates in adolescents (Christensen et al., 2010; Borrow et al., 2017). This explanation is strengthened by the significantly higher pre-vaccination rSBA geometric mean titer in the adolescents. Remarkably, the number of participants with protective pre-vaccination titers was not different between the two age groups. As a side note, this small difference might also partly be explained by polyreactive antibodies, either IgM, IgG or IgA, that can have antibacterial activity due to binding of distinct ligands, such as proteins, lipids and carbohydrates, without pathogen specificity (Notkins, 2004; Zhou et al., 2007). Since sharp reductions of these antibodies were found with age (Notkins, 2004), the adolescent study group might possess higher quantities of these polyreactive antibodies that might bind in small amounts to the bacterial polysaccharides and thereby possibly add to the explanation of the differences in the pre-vaccination IgG and IgM concentrations. However, the exact biological relevance of these antibodies is unknown (Notkins, 2004, Zhou et al., 2007).

Remarkably, a few individuals possessed sufficient specific

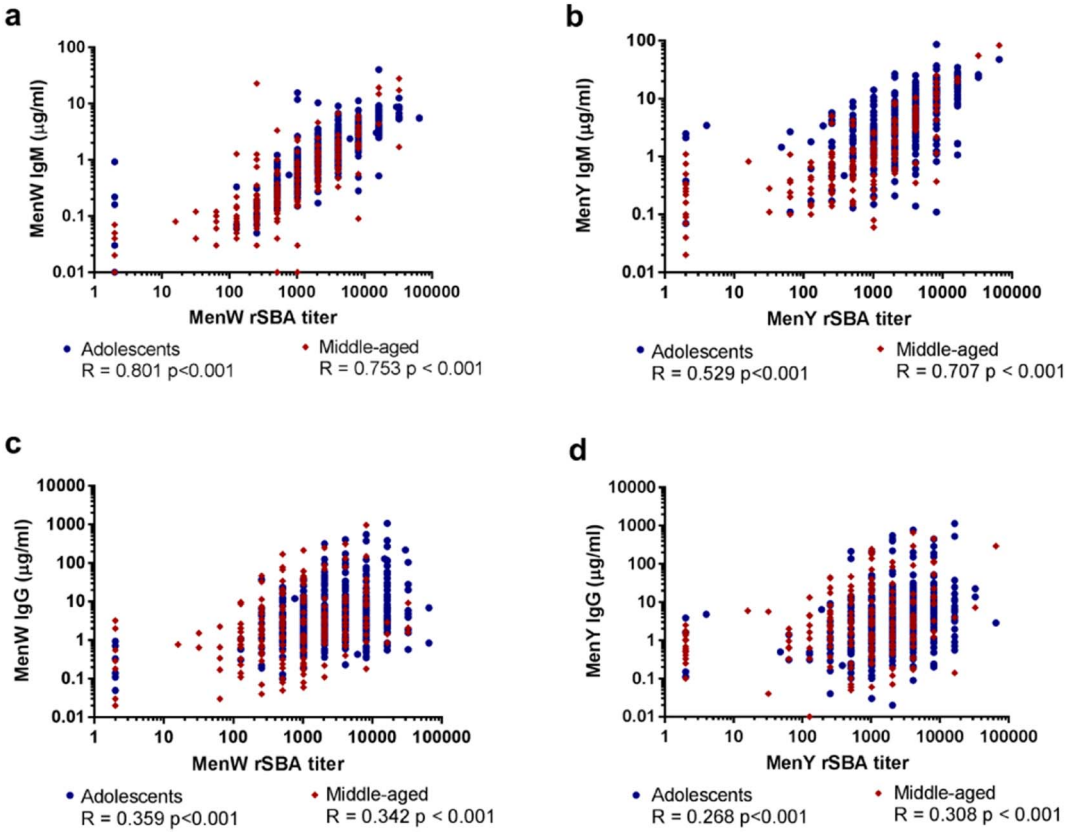

Fig. 5. Correlation between the post-vaccination rSBA titers with the IgM and IgG concentrations.
Correlation between all post-vaccination rSBA titers (both 28 days and 1 year) with the IgM concentrations for MenW **(a)**, and MenY **(b)**, as well as the IgG concentrations for MenW **(c)**, and MenY **(d)**. The adolescents are depicted in blue and the middle-aged adults in red. The correlations were determined with the Pearson's correlation, after log-transformation of all data.

meningococcal antibody concentrations which were not functional in the bacterial assay. Most likely these participants (mostly adults) produced enough specific anti-PS antibodies, but these antibodies fail in functionality of affinity or might be directed against less important epitopes of the polysaccharides, also seen for pneumococcal antibody levels in the elderly (Schenkein et al., 2008; Elberse et al., 2011).

Future studies will determine the long-term differences in the meningococcal antibody levels between the middle-aged adults and the adolescents. Nevertheless, based on the higher antibody decay rates 1 year post-vaccination, we hypothesize that these rates between the two age groups will diverge even further, likely due to limited niches available for long-lived plasma cells in the bone marrow of middle-aged adults. Of importance, since the antibody functionality was highly dependent on IgM responses, long-term protection by IgM is questionable. Follow-up samples will enlarge our understanding of the IgM responses and induction of memory B-cell responses on the antibody functionality after primary vaccination. Moreover, the immunogenicity of booster vaccination at elderly age is of interest for future study, after primary vaccination at middle-age. This booster vaccination might be positively influenced by the early induced pre-vaccination immunity, as also shown for other vaccines (Weinberger et al., 2013).

In conclusion, although protective functional antibodies are obtained in the middle-aged adults, the functional antibody titers after primary meningococcal vaccination over a 1 year time period are significantly lower as compared to adolescents. Most importantly, higher antibody decay was observed in the middle-aged adults, resulting in lower numbers of protected middle-aged adults 1 year post-vaccination. Large part of these differences between the adolescents and middle-aged adults was explained by a sharp drop in the IgM response. This reduced IgM response with age might indicate early signs of immune ageing already in the middle-aged adults. Consequently, these results are of importance for the development of future vaccine strategies for the ageing population.

Conflict of interest statement

MH, MR, MB, GB, and AMB declare no conflict of interest. AB is a consultant for Grunenthal Gmbh (Germany).

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Author contributions

MH and MR planned and performed the clinical work of the middle-aged adults and adolescents study respectively. MH, MR, and MB executed the laboratory experiments. MH performed the statistical analysis. MH, MR, GB, AB, and AMB conceived the study, interpreted the data and wrote the manuscript. All authors critically revised the

manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.exger.2017.12.014.

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